TAHC – SEPTEMBER 2005 REPORT

GUIDELINES FOR THE SURVEILLANCE OF BLUETONGUE

Article 3.X.X.1.

Introduction

This Appendix defines the principles and provides a guide for the surveillance of bluetongue (BT) in accordance with Appendix 3.8.1., applicable to countries seeking recognition for a declared BT status, with or without the use of vaccination. This may be for the entire country, *zone* or *compartment*. Guidance for countries seeking free status following an *outbreak* and for the maintenance of BT status is also provided. This Appendix complements Chapter 2.2.13.

BT is a vector-borne infection transmitted by different species of *Culicoides* insects in a range of ecosystems. An important component of BT epidemiology is vectorial capacity which provides a measure of disease risk that incorporates vector competence, abundance, biting rates, survival rates and extrinsic incubation period. However, methods and tools for measuring some of these vector factors remain to be developed, particularly in a field context. Therefore, surveillance for BT should focus on transmission in domestic ruminants.

Susceptible wild ruminant populations should be included in surveillance only if necessary for trade.

The impact and epidemiology of BT differ widely in different regions of the world and therefore it is impossible to provide specific guidelines for all situations. It is incumbent upon Member Countries to provide scientific data that explain the epidemiology of BT in the region concerned and adapt the surveillance strategies for defining their infection status (free, endemic or area of potential spread) to the local conditions. There is considerable latitude available to Member Countries to justify their infection status at an acceptable level of confidence.

Surveillance for BT should be in the form of a continuing programme.

Article 3.X.X.2.

Case definition

For the purposes of surveillance, a *case* refers to an animal infected with BT virus (BTV).

For the purposes of *international trade*, a difference must be made between a case as defined below and an animal that is potentially infectious to vectors. The conditions for trade are defined in Chapter 2.2.13 of the *Terrestrial Code*.

The purpose of surveillance is the detection of virus circulation in a country or *zone* and not the status of an individual animal or herds. Surveillance deals not only with the occurrence of clinical signs caused by BTV, but also with the presence of infection with BTV in the absence of clinical signs.

The following defines the occurrence of BTV infection:

1. BTV has been isolated and identified as such from an animal or a product derived from that animal, or

viral antigen or viral RNA specific to one or more of the serotypes of BTV has been identified in samples from one or more animals showing clinical signs consistent with BT, or epidemiologically linked to a confirmed or suspected *case*, or giving cause for suspicion of previous association or contact with BTV, or

2. antibodies to structural or nonstructural proteins of BTV that are not a consequence of vaccination have been identified in one or more animals showing clinical signs consistent with BT, or epidemiologically linked to a confirmed or suspected *case*, or giving cause for suspicion of previous association or contact with BTV.

Standards for diagnostic tests and vaccines are described in the *Terrestrial Manual.*

Article 3.X.X.3.

General conditions and methods

1. A surveillance system in accordance with Appendix 3.8.1. should be under the responsibility of the *Veterinary Administration*. In particular:

   a) a formal and ongoing system for detecting and investigating *outbreaks of disease* should be in place;

   b) a procedure should be in place for the rapid collection and transport of samples from suspect cases of BT to a laboratory for BT diagnosis as described in the *Terrestrial Manual*;

   c) a system for recording, managing and analysing diagnostic and surveillance data should be in place.

2. The BT surveillance programme should:

   a) include an early warning system for reporting suspicious cases. Farmers and workers, who have day-to-day contact with domestic ruminants, as well as diagnosticians, should report promptly any suspicion of BT to the *Veterinary Authority*. They should be supported directly or indirectly (e.g. through private *veterinarians* or *veterinary para-professionals*) by government information programmes and the *Veterinary Administration*. An effective surveillance system will periodically identify suspicious cases that require follow up and investigation to confirm or exclude that the cause of the condition is BTV. The rate at which such suspicious cases are likely to occur will differ between epidemiological situations and cannot therefore be predicted reliably. All suspected cases of BT should be investigated immediately and samples should be taken and submitted to an *approved laboratory*. This requires that sampling kits and other equipment are available for those responsible for surveillance;

   b) conduct random or targeted serological and virological surveillance appropriate to the infection status of the country or *zone*.

With regards to BT, *compartment* refers to *establishments* where animals are kept in a confirmed vector free environment to prevent BTV infection. Generally, the conditions to prevent exposure of susceptible animals to BTV infected vectors will be difficult to apply. However, under specific situations like *artificial insemination centres* or *quarantine stations* such conditions may be met. The testing requirements for animals kept in these facilities are described in Articles 2.2.13.11 and 2.2.13.15.

Article 3.X.X.4.

Surveillance strategies

The target population for surveillance aimed at identification of *disease* and/or *infection* should cover susceptible domestic ruminants within the country, *zone* or *compartment*. Active and passive surveillance for BTV infection should be ongoing. Surveillance should be composed of random or targeted approaches using virological, serological and clinical methods appropriate for the infection status of the country or *zone*.

The strategy employed may be based on randomised sampling requiring surveillance consistent with demonstrating the absence of BTV infection at an acceptable level of confidence. The frequency of sampling should be dependent on the epidemiological situation. Random surveillance is conducted using serological tests described in the *Terrestrial Manual*. Positive serological results may be followed up with virological methods as appropriate.

Targeted surveillance (e.g. based on the increased likelihood of *infection* in particular localities or species) may be an appropriate strategy. Virological and serological methods may be used concurrently to define the BTV status of targeted populations.

A country should justify the surveillance strategy chosen as being adequate to detect the presence of BTV infection in accordance with Appendix 3.8.1. and the prevailing epidemiological situation. It may, for example, be appropriate to target clinical surveillance at particular species likely to exhibit clinical signs (e.g. sheep). Similarly, virological and serological testing may be targeted to species that rarely show clinical signs (e.g. cattle).

In vaccinated populations, serological and virological surveillance is necessary to detect the BTV types circulating to ensure that all circulating types are included in the vaccination programme.

If a Member Country wishes to declare freedom from BTV infection in a specific *zone*, the design of the surveillance strategy would need to be aimed at the population within the *zone*.

For random surveys, the design of the sampling strategy will need to incorporate epidemiologically appropriate design prevalence. The sample size selected for testing will need to be large enough to detect *infection* if it were to occur at a predetermined minimum rate. The sample size and expected prevalence determine the level of confidence in the results of the survey. The applicant country must justify the choice of design prevalence and confidence level based on the objectives of surveillance and the epidemiological situation, in accordance with Appendix 3.8.1. Selection of the design prevalence in particular needs to be based on the prevailing or historical epidemiological situation.

Irrespective of the survey approach selected, the sensitivity and specificity of the diagnostic tests employed are key factors in the design, sample size determination and interpretation of the results obtained. Ideally, the sensitivity and specificity of the tests used should be validated for the vaccination/infection history and the different species in the target population.

Irrespective of the testing system employed, surveillance system design should anticipate the occurrence of false positive reactions. If the characteristics of the testing system are known, the rate at which these false positives are likely to occur can be calculated in advance. There needs to be an effective procedure for following up positives to ultimately determine with a high level of confidence, whether they are indicative of infection or not. This should involve both supplementary tests and follow-up investigation to collect diagnostic material from the original sampling unit as well as those which may be epidemiologically linked to it.

The principles involved in surveillance for *disease*/*infection* are technically well defined. The design of surveillance programmes to prove the absence of BTV infection/circulation needs to be carefully followed to avoid producing results that are either insufficiently reliable to be accepted by the OIE or international trading partners, or excessively costly and logistically complicated. The design of any surveillance programme, therefore, requires inputs from professionals competent and experienced in this field.

1. Clinical surveillance

Clinical surveillance aims at the detection of clinical signs of BT at the flock/herd level. Whereas significant emphasis is placed on the diagnostic value of mass serological screening, surveillance based on clinical inspection should not be underrated, particularly during a newly introduced infection. In sheep and occasionally goats, clinical signs may include oedema, hyperaemia of mucosal membranes, coronitis and cyanotic tongue.

BT suspects detected by clinical surveillance should always be confirmed by laboratory testing.

2. Serological surveillance

An active programme of surveillance of host populations to detect evidence of BTV transmission is essential to establish BTV status in a country or *zone*. Serological testing of ruminants is one of the most effective methods of detecting the presence of BTV. The species tested depends on the epidemiology of BTV infection, and the species available, in the local area. Cattle are usually the most sensitive indicator species.

Surveillance may include serological surveys, for example abattoir surveys, the use of sentinel animals, or a combination of methods.

The objective of serological surveillance is to detect antibodies against BTV using tests prescribed in the *Terrestrial Manual*. Positive BTV antibody tests results can have four possible causes:

a) natural infection with BTV,

b) vaccination against BTV,

c) maternal antibodies,

d) positive results due to the lack of specificity of the test.

It may be possible to use sera collected for other survey purposes for BTV surveillance. However, the principles of survey design described in these guidelines and the requirements for a statistically valid survey for the presence of BTV infection should not be compromised.

The results of random or targeted serological surveys are important in providing reliable evidence that no BTV infection is present in a *country*, *zone* or *compartment*. It is, therefore, essential that the survey is thoroughly documented.

Serological surveillance in a free *zone* should target those areas that are at highest risk of BTV transmission, based on the results of previous surveillance and other information. This will usually be towards the boundaries of the free *zone*. In view of the epidemiology of BTV infection, either random or targeted sampling is suitable to select herds and/or animals for testing.

A surveillance *zone* within a free country or *zone* should separate it from a potentially infected country or *zone*. Serological surveillance in a free country or *zone* should be carried out over an appropriate distance from the border with a potentially infected country or *zone*, based upon geography, climate, history of infection and other relevant factors.

Serological surveillance in infected *zones* will identify changes in the boundary of the *zone*, and can also be used to identify the BTV types circulating. In view of the epidemiology of BTV infection, either random or targeted sampling is suitable.

3. Virological surveillance

Isolation and genetic analysis of samples of BTV from a proportion of infected animals is beneficial in terms of providing information on serotype and genetic characteristics of the viruses concerned.

Virological surveillance using tests described in the *Terrestrial Manual* can be conducted:

a) to identify virus circulation in at risk populations,

b) to confirm clinically suspect cases,

c) to follow up positive serological results,

d) to better characterize the genotype of circulating virus in a country or *zone*.

4. Sentinel herds

Sentinel herds are a form of targeted surveillance with a prospective study design. They are the preferred strategy for BTV surveillance. They comprise groups of unexposed animals managed at fixed locations and sampled regularly to detect new BTV infections.

The primary purpose of a sentinel herd programme is to detect BTV infections occurring at a particular place, for instance sentinel groups may be located on the usual boundaries of infected *zones* to detect changes in distribution of BTV. In addition, sentinel herd programmes allow incidence rates to be determined and the timing of infections to be observed.

A sentinel herd programme should use animals of known source and history of exposure, control management variables such as use of insecticides and be flexible in its design in terms of sampling frequency and choice of tests.

Care is necessary in choosing the sites for the sentinel groups. The aim is to maximise the chance of detecting BTV activity at the geographical location for which the sentinel site acts as a sampling point. The effect of secondary factors that may influence events at each location, such as climate, may also be analysed. To avoid confounding factors, sentinel groups should comprise animals selected to be of similar age and susceptibility to BTV infection. Cattle are the most appropriate sentinels but other domestic ruminant species may be used. The only feature distinguishing groups of sentinels should be their geographical location.

Sera from sentinel herd programmes should be stored methodically in a serum bank to allow retrospective studies to be conducted in the event of new serotypes being isolated.

The frequency of sampling will depend on the reason for choosing the sampling site. In endemic areas, virus isolation will allow monitoring of the serotypes and genotypes of BTV circulating during each time period. The borders between infected and non infected areas can be defined by serological detection of infection. Monthly sampling intervals are frequently used. Sentinels in declared free *zones* add to confidence that BTV infections are not occurring unobserved. In such cases, sampling prior to and after the possible period of transmission is sufficient.

The definitive measure of a country or *zone*'s BTV infection status is detection and identification of the viruses. If virus isolation is required, sentinels should be sampled at sufficiently frequent intervals to ensure that samples are collected during the period of viraemia.

5. Vector surveillance

BTV is transmitted between ruminant hosts by vector species of *Culicoides* which vary across the world. It is therefore important to be able to identify potential vector species accurately although many such species are closely related and difficult to differentiate with certainty.

The main purpose of vector surveillance is to define high, medium and low-risk areas and local details of seasonality by determining the species present in an area, their seasonal incidence and profile, and their abundance. Vector surveillance has particular relevance to potential areas of spread. Long term surveillance can also be used to assess vector abatement measures.

The most effective way of gathering this information should take account of the biology and behavioural characteristics of the local vector species of *Culicoides* and may include the use of Onderstepoort-type light traps or similar, operated from dusk to dawn in locations adjacent to domestic ruminants, or the use of drop traps over ruminant animals.

The number of traps to be used in a vector surveillance system and the frequency of their use will depend on the availability of resources but is also dependent upon the size or ecological characteristics of the area to be surveyed.

The operation of vector surveillance sites at the same locations as sentinel herds is advisable.

The use of a vector surveillance system to detect the presence of circulating virus is not recommended as a routine procedure as the typically low vector infection rates mean that such detections can be rare. Other surveillance strategies (e.g. the use of sentinel herds of domestic ruminants) are preferred to detect virus circulation.

Article 3.X.X.5.

Documentation of BTV infection free status

1. Countries declaring freedom from BTV infection for the country, *zone* or compartment

In addition to the general conditions described in Chapter 2.2.13. of the *Terrestrial Code*, a Member Country declaring freedom from BTV infection for the entire country, or a *zone* or a *compartment* should provide evidence for the existence of an effective surveillance programme. The strategy and design of the surveillance programme will depend on the prevailing epidemiological circumstances and should be planned and implemented according to general conditions and methods described in this Appendix, to demonstrate absence of BTV infection during the preceding 24 months in susceptible domestic ruminant populations. This requires the support of a laboratory able to undertake identification of BTV infection through virus detection and antibody tests described in the *Terrestrial Manual*. This surveillance should be targeted to non-vaccinated animals. Clinical surveillance may be effective in sheep while serological surveillance is more appropriate in cattle.

2. Additional requirements for countries, *zones* or compartments that practise vaccination

Vaccination to prevent the transmission of BTV may be part of a disease control programme. The level of flock or herd immunity required to prevent transmission will depend on the flock or herd size, composition (e.g. species) and density of the susceptible population. It is therefore impossible to be prescriptive. The vaccine must also comply with the provisions stipulated for BTV vaccines in the *Terrestrial Manual*. Based on the epidemiology of BTV infection in the country, *zone* or *compartment*, it may be that a decision is reached to vaccinate only certain species or other subpopulations.

In countries or *zones* that practice vaccination there is a need to perform virological and serological tests to ensure the absence of virus circulation. These tests should be performed on non-vaccinated subpopulations or on sentinels. The tests have to be repeated at appropriate intervals according to the purpose of the surveillance programme. For example, longer intervals may be adequate to confirm endemicity, while shorter intervals may allow on-going demonstration of absence of transmission.

Article 3.X.X.6.

The use and interpretation of serological and virus detection tests

1. Serological testing

Ruminants infected with BTV produce antibodies to structural and non-structural viral proteins, as do animals vaccinated with current modified live virus vaccines. Antibodies to the BTV serogroup antigen are detected with high sensitivity and specificity by competitive ELISA (c-ELISA) and to a lesser extent by AGID as described in the *Terrestrial Manual*. Positive c-ELISA results can be confirmed by neutralization assay to identify the infecting serotype (s), however BTV infected ruminants can produce neutralizing antibodies to serotypes of BTV other than those to which they were exposed (false positive results), especially if they have been infected with multiple serotypes.

2. Virus detection

The presence of BTV in ruminant blood and tissues can be detected by virus isolation or polymerase chain reaction (PCR) as described in the *Terrestrial Manual*.

Interpretation of positive and negative results (both true and false) differs markedly between these tests because they detect different aspects of BTV infection, specifically (1) infectious BTV (virus isolation) and (2) nucleic acid (PCR). The following are especially relevant to interpretation of PCR assays:

a) The nested PCR assay detects BTV nucleic acid in ruminants long after the clearance of infectious virus. Thus positive PCR results do not necessarily coincide with active infection of ruminants. Furthermore, the nested PCR assay is especially prone to template contamination, thus there is considerable risk of false positive results.

b) PCR procedures other than real time PCR allow sequence analysis of viral amplicons from ruminant tissues, insect vectors or virus isolates. These sequence data are useful for creating data bases to facilitate important epidemiological studies, including the possible distinction of field and vaccine virus strains of BTV, genotype characterization of field strains of BTV, and potential genetic divergence of BTV relevant to vaccine and diagnostic testing strategies.

It is essential that BTV isolates are sent regularly to the OIE Reference Laboratories for genetic and antigenic characterization.

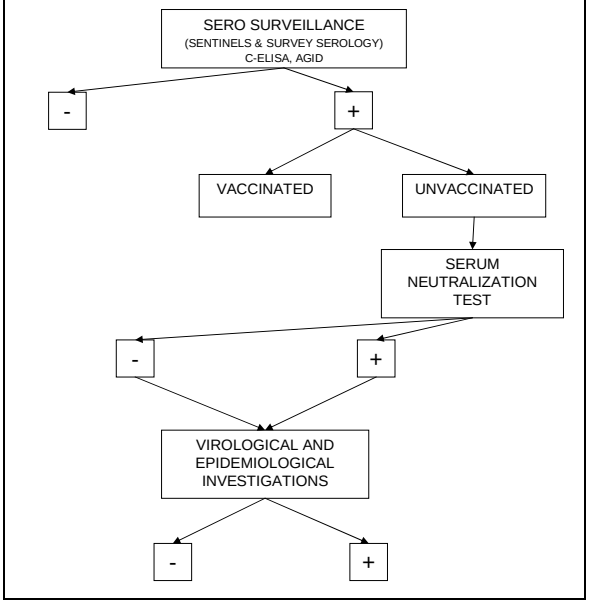

Figure 1

Application of laboratory tests in serological surveillance

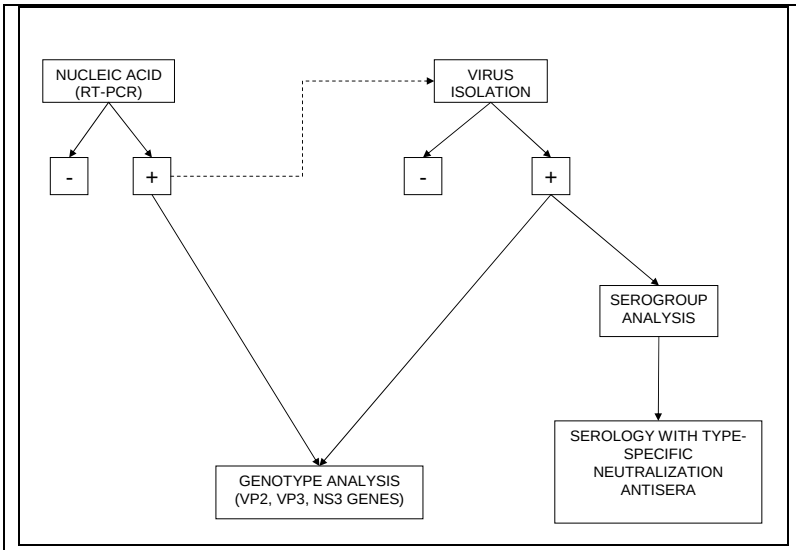

Figure 2

Application of laboratory tests in virological surveillance